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STUDY TITLE: Local Lymph Node Assay (LLNA) in Mice

TEST GUIDELINES: U.S. EPA Health Effects Test Guidelines
OPPTS 870.2600 (2003)

OECD Guideline for the Testing of Chemicals
Section 4 (Part 429) (2002)

AUTHOR:

ORIGINAL REPORT

COMPLETED: October 19, 2010

REPORT REVISION 1

COMPLETED: November 29, 2010

PERFORMING LABORATORY:

LABORATORY PROJECT ID:

WORK REQUEST NUMBER:

SERVICE CODE NUMBER:

SPONSOR:

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards, which are compatible with current OECD Good Laboratory Practices, except for the items documented below. None of the items listed impact the validity of the study.

1. The test substance was characterized by the sponsor prior to the initiation of this study. Although the characterization was not performed under Good Laboratory Practice Standards, the accuracy of the data is considered sufficient for the purposes of this study.
2. The test substance and control preparations used in the study were not analyzed for concentration, uniformity, or stability. The procedures used by trained staff to prepare the dosing preparations ensured:
 - the accuracy of concentration because all preparations were performed using calibrated pipettes,
 - uniformity and stability because each preparation was formulated daily just prior to dosing, and
 - each vehicle and positive control group gave expected results in the study.
3. An in-life phase inspection was not performed by Quality Assurance. This did not affect the validity of the study because this type of study is conducted by trained personnel and is routinely inspected by Quality Assurance.

Study Director:

29 Nov 2010
Date

QUALITY ASSURANCE STATEMENT

Work Request Number:
Service Code Number:

Key inspections for the above referenced study were completed by the Quality Assurance Unit of
and the findings were submitted on the following dates:

<i>Audit Dates</i>	<i>Date Reported to Study Director</i>	<i>Date Reported to Management</i>
<u>Protocol:</u> September 23, 2010	September 23, 2010	September 23, 2010
<u>Report/Records:</u> October 06-08, 2010	October 08, 2010	October 12, 2010
<u>Report Revision 1:</u> November 23, 2010 November 29, 2010	November 23, 2010 November 29, 2010	November 23, 2010 November 29, 2010

Reported by:

29-NOV-2010

Date

CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

Reviewed by:

29-NOV-2010
Date

Issued by Study Director:

29-NOV-2010
Date

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STUDY INFORMATION

Substance Tested:

Number:

Composition:

Purity: See composition, above

Physical Characteristics:

Stability: The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.

Study Initiated/Completed: September 7, 2010 / (see report cover page)

Experimental Start/Termination: September 8, 2010 / September 14, 2010

In-Life Initiated/Completed: September 8, 2010 / September 13, 2010

Notebook Number(s):

REASON FOR REVISION 1

The Study Information page was revised and the Certificate of Analysis was removed from the report, at the request of the sponsor.

SUMMARY

The objective of this study was to evaluate the potential of _____ to produce a dermal sensitization response in mice using the local lymph node assay (LLNA). Five groups of 5 female CBA/JHsd mice were dosed for 3 consecutive days with 0% (vehicle control), 5%, 25%, 50%, or 100% _____ on both ears. Dimethylsulfoxide (DMSO) was used as the diluting vehicle. One group of 5 female mice was dosed for 3 consecutive days with 25% hexylcinnamaldehyde (HCA) in DMSO as a positive control. On test day 5 of the assay, mice received ³H-thymidine by tail vein injection and were sacrificed approximately 5 hours later. The cell proliferation in the draining auricular lymph nodes of the ears from the test substance groups was then evaluated and compared to the vehicle control group.

No test substance-related changes in mean body weights were observed at any test concentration. No clinical signs of toxicity were observed in the study.

No statistically significant increases in cell proliferation measurements compared to the vehicle control group were observed at any test concentration. Stimulation indices (SIs) of less than 3.0 were observed at all test concentrations of _____. Therefore, the EC3 value (the estimated concentration required to induce a threshold positive response, i.e., SI = 3) for the test substance under the conditions of this study was not calculable. A 25% concentration of the positive control, HCA, produced a dermal sensitization response in mice. Therefore, the LLNA test system was valid for this study with _____. Under the conditions of this study, _____ did not produce a dermal sensitization response in mice.

Based on these data, _____ is not a dermal sensitizer in mice.

INTRODUCTION

The purpose of this study was to examine the dermal sensitization potential of _____ using the mouse local lymph node assay (LLNA).^(1,2,3,4,5) Following the topical application of the test substance to the dorsal side of both ears, the dermal sensitization potential of the test substance was evaluated by measuring the proliferation of lymphocytes (via radiolabel uptake) obtained from the auricular lymph nodes (i.e., the lymph nodes that drain the ears). Results were compared to the vehicle control group. The dermal route was selected because it is a potential route of human exposure to the test substance.

_____ is a liquid and did not appear to have severe skin-irritating capability (pH ~10). The 100% concentration (neat test substance) was chosen as the high dose. For subsequent concentrations, the test substance was prepared in dimethylsulfoxide (DMSO).

ANIMAL WELFARE ACT COMPLIANCE

This study complied with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR) and the Guidelines from the Guide for the Care and Use of Laboratory Animals (NRC 1996). All studies conducted by or for _____ adhere to the following principles:

- The sponsor and/or the study director ensure that the study described in this report does not unnecessarily duplicate previous experiments, and is in compliance with the _____ Policy on Animal Testing.
- Whenever possible, procedures used in this study have been designed to implement a reduction, replacement, and/or refinement in the use of animals in an effort to avoid or minimize discomfort, distress or pain to animals. All methods are described in this study report or in written laboratory standard operating procedures.
- _____ policy is that animals experiencing severe pain or distress that cannot be relieved are painlessly euthanized, as deemed appropriate by the veterinary staff and study director or appropriate designee.
- Methods of euthanasia used during this study were in conformance with the above referenced regulation and the recommendations of the American Veterinary Medical Association (AVMA), 2007 Guidelines on Euthanasia.
- Animals were provided with species-appropriate environmental enrichment.
- _____ is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

STUDY DESIGN

The study design was as follows:

Group	Number/ Group	Dosage (%) ^a
1	5	0 (Vehicle Control)
2	5	5
3	5	25
4	5	50
5	5	100
6	5	25 (Positive Control)

a % = percent of test substance in vehicle control (e.g., 100% = 1 g/mL, or neat test substance)

Study Parameter	Frequency
Body Weight	Test days 0 and 5
Daily Animal Health Observations	At least once daily
Careful Clinical Observations	Prior to dosing and prior to sacrifice
Dosing	Test days 0-2
Days of Rest	Test days 3-4
Injection of Radioactivity	Test day 5
Removal of Lymph Nodes	At sacrifice (test day 5)
Disintegrations per minute (dpm) data	Test day 6

MATERIALS AND METHODS

A. Test Guidelines

The study design complied with the following test guidelines:

- U.S. EPA, OPPTS 870.2600: Skin Sensitization, *Health Effects Test Guidelines* (2003)
- OECD, Section 4 (Part 429): Skin Sensitisation: Local Lymph Node Assay, *Guideline for the Testing of Chemicals* (2002)

B. Vehicle Control

The vehicle control, DMSO, was purchased commercially and used for all test substance dilutions on all dose days. Impurities in the vehicle control were not expected to interfere with the study results. The vehicle control was assumed to be stable under the conditions of the study. No evidence of instability, such as a change in color or physical state, was observed.

C. Test Substance

The test substance, _____ was supplied by the sponsor as an amber liquid. The test substance used for this study was assigned _____ number _____. The sample was stored according to the sponsor's instructions. The test substance appeared to be stable under the conditions of the study. No evidence of instability, such as a change in color or physical state, was observed.

The test substance was prepared in the vehicle control according to the concentrations listed in the Study Design, except for the 100% concentration, which was used neat.

D. Positive Control

The positive control, hexylcinnamaldehyde (HCA), was purchased commercially. Any available information on the positive control was included in the study records. Impurities in the positive control were not expected to interfere with the study results. The positive control appeared to be stable under the conditions of the study. No evidence of instability, such as a change in color or physical state, was observed.

A 25% HCA solution in the vehicle control was blended using a vortex mixer and stored in a vial protected from light until dosing was completed.

E. Dosing Preparations and Analyses

Prior to study start, a quantity of the test substance was evaluated for solubility in a particular vehicle. The control and test substance concentrations and method of preparation were based on solubility information. All dose preparations were formulated fresh daily.

Dose preparations were not analyzed for homogeneity or accuracy of concentration. The dose preparation procedures were believed to provide homogeneous mixtures at the targeted concentrations. In the absence of visible change in color or physical state, all dose preparations were assumed to be stable throughout the study.

All dose preparations applied to the test site were assumed to be available for absorption by the test system unless otherwise indicated in the study records. All calculations and the evaluation of effects were based on the applied dose.

F. Test System

Female (nulliparous and non-pregnant) CBA/JHsd mice were received from Harlan Sprague Dawley, Frederick, Maryland, U.S.A.

The CBA/JHsd mouse was selected to conduct the LLNA because it is the strain recommended in the test guidelines. In addition, _____ has extensive LLNA experience with the CBA/JHsd mouse strain, and this strain has undergone extensive interlaboratory validation with the LLNA.^(1,2,3,4,5)

G. Animal Husbandry**1. Housing**

All animals were housed in solid bottom cages with appropriate bedding and nestlets toys as enrichment. During quarantine, animals were housed in pairs. After assignment to groups, and during the in-life phase of the study, animals were housed singly.

2. Environmental Conditions

Animal rooms were maintained at a temperature of 18-26°C (64-79°F) and a relative humidity of 30-70%. Animal rooms were artificially illuminated (fluorescent light) on an approximate 12-hour light/dark cycle. Any excursions outside of these ranges were of insufficient magnitude and/or duration to have adversely affected the validity of the study.

3. Feed and Water

All mice were provided tap water *ad libitum*. All mice were fed PMI[®] Nutrition International, LLC Certified Rodent LabDiet[®] 5002 *ad libitum*.

4. Animal Health and Environmental Monitoring Program

As specified in the animal health and environmental monitoring program, the following procedures are performed periodically to ensure that contaminant levels are below those that would be expected to impact the scientific integrity of the study:

- Water samples are analyzed for total bacterial counts, and the presence of coliforms, lead, and other contaminants.
- Samples from freshly washed cages and cage racks are analyzed to ensure adequate sanitation by the cagewashers.

Certified animal feed is used, guaranteed by the manufacturer to meet specified nutritional requirements and not to exceed stated maximum concentrations of key contaminants, including specified heavy metals, aflatoxin, chlorinated hydrocarbons, and organophosphates. The presence of these contaminants below the maximum concentration stated by the manufacturer would not be expected to impact the integrity of the study.

The animal health and environmental monitoring program is administered by the attending laboratory animal veterinarian. Evaluation of these data did not indicate any conditions that affected the validity of the study.

H. Acclimation Period

Upon arrival at all mice were:

- quarantined for a minimum of 6 days.

- identified temporarily by the presence or absence of a colored tail mark and cage identification.
- observed with respect to weight gain and any gross signs of disease or injury.

The mice were released from quarantine based on body weights and clinical signs of all mice.

I. Assignment to Groups

Mice, selected based on adequate body weight gain and freedom from any ear abnormalities (e.g., torn, scratched) or clinical signs of disease or injury, were distributed into study groups as designated in the Study Design. Prior to study start, each mouse was assigned to a group using a randomly generated, computer-based algorithm such that individual pretest body weights did not vary more than 20% of the group mean.

At grouping, each mouse was assigned an identification number. The identification number was marked on the tail of each mouse with solvent-resistant ink. Color-coded labels were attached to the animal rack above each cage prior to dosing and included the group number, the animal number, the dose concentration, and the _____ number.

At study start (test day 0), mice were approximately 9 weeks old and weighed between 21.8 and 23.1 grams.

Mice not assigned to a test group were released for other laboratory purposes or sacrificed by isoflurane anesthesia followed by carbon dioxide asphyxiation and discarded without anatomic pathology evaluation, at the discretion of the study director.

J. Body Weights

All mice were weighed on test day 0 and prior to sacrifice on test day 5.

K. Clinical Observations

Daily animal health observations to detect moribund or dead mice and abnormal behavior and appearance among mice were conducted at least once daily throughout the study. Careful clinical observations were performed on test day 0, prior to each dose (at approximately the same time \pm 2 hours) on test day 1 and 2, and on the day of sacrifice by individually handling and examining each animal for abnormal behavior and appearance.

L. Local Lymph Node Assay

Twenty-five μ L of vehicle control, _____ or positive control were administered topically to the dorsum of each mouse ear for 3 consecutive days (test days 0-2) at concentrations listed in the Study Design. Test days 3-4 were days of rest followed by intravenous injection of 20 μ Ci of 3 H-thymidine in PBS per mouse on test day 5.

Approximately 5 hours after the injection, animals were sacrificed by isoflurane anesthesia followed by carbon dioxide asphyxiation, draining auricular lymph nodes were removed, and

single cell suspensions were prepared. The single cell suspensions were incubated at 2-8°C overnight. On test day 6, the single cell suspensions were counted on a beta counter and reported as disintegrations per minute (dpm).

M. Data Analysis and Interpretation of Results

A stimulation index (SI) was derived for each experimental group by dividing the mean dpm of each experimental group by the mean dpm of the vehicle control group. The decision process in regard to a positive response includes an SI of greater than or equal to 3.0 together with consideration of dose response and, where appropriate, statistical significance.

When possible, an EC3 value for the SI data was derived from linear interpolation of points on the dose-response curve immediately above and below the 3-fold threshold. The equation used for calculation of EC3 was:

$$EC3 = c + [(3 - d)/(b - d)] \times (a - c)$$

where:

- a = the lowest concentration giving stimulation greater than 3
- b = the actual SI caused by a
- c = the highest concentration failing to produce an SI of 3
- d = the actual SI caused by c

The European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) has recommended categories classifying contact allergens based on the EC3 values as listed in the table below:⁽⁶⁾

Category	EC3 Value (%)
Extreme	<0.1
Strong	≥0.1 - <1
Moderate	≥1 - <10
Weak	≥10 - ≤100

STATISTICAL ANALYSES

Significance was judged at $p < 0.01$. Lymph node dpm data were transformed to Log to obtain normality or homogenous variances.

Parameter	Preliminary Test	Method of Statistical Analysis	
		If preliminary test is not significant	If preliminary test is significant
Lymph Node dpm Data ^a	Test for lack of trend ⁽⁷⁾	Sequential application ⁽⁸⁾ of the Jonckheere-Terpstra trend test ⁽⁹⁾	Preliminary tests for pairwise comparison
	OR ^b		
	Levene's test for homogeneity ⁽¹⁰⁾ and Shapiro-Wilk test ⁽¹¹⁾ for normality ^c	One-way analysis of variance ⁽¹²⁾ followed by Dunnett's test ^(13,14,15)	Kruskal-Wallis test ⁽¹⁶⁾ followed by Dunn's test ⁽¹⁷⁾

- a Positive control data were not included in the statistical analysis of the test substance groups.
- b Pairwise comparisons and associated preliminary tests were only conducted if the test for lack of trend was significant.
- c If the Shapiro-Wilk test was not significant but Levene's test was significant, a robust version of Dunnett's test was used. If the Shapiro-Wilk test was significant, Kruskal-Wallis test was followed by Dunn's test.

RESULTS AND DISCUSSION

A. Body Weights and Clinical Signs of Toxicity

(Table 1, Appendices A-B)

No test substance-related changes in mean body weights were observed at any test concentration. No clinical signs of toxicity were observed in the study.

B. Stimulation Index Data

(Table 2, Appendix C)

No statistically significant increases in cell proliferation measurements compared to the vehicle control group were observed at any test concentration. SIs of less than 3.0 were observed at all test concentrations of . Therefore, the EC3 value (the estimated concentration required to induce a threshold positive response, i.e., $SI = 3$) for the test substance under the conditions of this study was not calculable. A 25% concentration of the positive control, HCA, produced a dermal sensitization response in mice. Therefore, the LLNA test system was valid for this study with . Under the conditions of this study, did not produce a dermal sensitization response in mice.

CONCLUSIONS

Based on these data, is not a dermal sensitizer in mice.

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, the protocol, amendments (if any), and the final report will be retained at

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TABLES

TABLES

EXPLANATORY NOTES

ABBREVIATIONS:

Summary of Clinical Observations

Stimulation Index Data

- dpm - disintegrations per minute
- n - number of animals evaluated
- N/A - not applicable
- S.D. - standard deviation
- SI - stimulation index

Table 1
Summary of Clinical Observations

Day numbers relative to Start Date						
Sex: Female	0% DMSO	5%	25%	50%	100%	25% HCA
Animal Count	5	5	5	5	5	5
Scheduled sacrifice						
Number of Observations	5	5	5	5	5	5
Number of Animals	5	5	5	5	5	5
Days from - to	5 5	5 5	5 5	5 5	5 5	5 5

Table 2
Stimulation Index Data

GROUP	MATERIAL TESTED	n	MEAN (dpm)	S.D. (dpm)	SI
1	0% Vehicle Control	5	897.50	299.74	N/A
2	5%	5	749.70	188.79	0.84
3	25%	5	1076.50	273.31	1.20
4	50%	5	1068.30	770.40	1.19
5	100%	5	768.10	336.18	0.86
6	25% Positive Control ^a	5	6627.10	1434.09	7.38

a Data were not included in the statistical analysis of the test substance groups.

Statistically significant increase in dpm data from vehicle control at $p < 0.01$ by Jonckheere-Terpstra trend test.

* Statistically significant increase in dpm data from vehicle control at $p < 0.01$ by Dunnett/Tamhane-Dunnett test.

@ Statistically significant increase in dpm data from vehicle control at $p < 0.01$ by Dunn's test.

~ Due to lack of control group values or variability among group means, statistical analyses were unable to be performed.

APPENDICES

Appendix A
Individual Body Weights

INDIVIDUAL BODY WEIGHTS

EXPLANATORY NOTES

ABBREVIATIONS:

g - gram
N - number of values included in calculation
S.D. - standard deviation

Local Lymph Node Assay (LLNA) in Mice

Individual Body Weights

Bodyweight (g)				

Day numbers relative to Start Date				
Group	Sex	Animal	0	5

1	f	101	22.4	22.5
		102	22.7	23.1
		103	21.9	21.3
		104	22.8	22.7
		105	23.1	22.9
		-----	-----	-----
		Mean	22.58	22.50
		S.D.	0.45	0.71
	N	5	5	
2	f	201	22.1	21.9
		202	22.5	22.9
		203	23.1	22.9
		204	22.9	24.7
		205	22.6	22.9
		-----	-----	-----
		Mean	22.64	23.06
		S.D.	0.38	1.01
	N	5	5	
3	f	301	22.1	22.7
		302	21.8	22.9
		303	22.7	23.3
		304	22.9	23.0
		305	22.9	22.5
		-----	-----	-----
		Mean	22.48	22.88
		S.D.	0.50	0.30
	N	5	5	

Nominal Dose:	Group 1 - 0% Vehicle Control	Group 2 - 5%	Group 3 - 25%	
	Group 4 - 50%	Group 5 - 100%	Group 6 - 25% Positive Control	

Local Lymph Node Assay (LLNA) in Mice

Individual Body Weights

Bodyweight (g)				

Day numbers relative to Start Date				
Group	Sex	Animal	0	5

4	f	401	22.0	23.1
		402	22.8	22.7
		403	22.6	22.7
		404	23.1	22.3
		405	22.1	21.9
		-----	-----	-----
		Mean	22.52	22.54
		S.D.	0.47	0.46
		N	5	5
5	f	501	21.8	21.6
		502	22.9	23.0
		503	22.1	22.9
		504	22.6	22.4
		505	23.1	22.9
		-----	-----	-----
		Mean	22.50	22.56
		S.D.	0.54	0.59
		N	5	5
6	f	601	23.0	22.1
		602	22.6	23.4
		603	22.7	22.6
		604	22.3	21.4
		605	21.9	22.9
		-----	-----	-----
		Mean	22.50	22.48
		S.D.	0.42	0.77
		N	5	5
Nominal Dose: Group 1 - 0% Vehicle Control Group 2 - 5% Group 3 - 25%				
Group 4 - 50% Group 5 - 100% Group 6 - 25% Positive Control				

Appendix B
Individual Clinical Observations and Mortality Records

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS

EXPLANATORY NOTES

ABBREVIATIONS:

X - present

NOTES:

Clinical observations and fates are recorded in the following time slots:

A - careful (pretreatment) and mode of death

Local Lymph Node Assay (LLNA) in Mice

Individual Clinical Observations and Mortality Records

Day numbers relative to Start Date								
Group	Sex	Animal	Clinical Sign	Site	0 A	1 A	2 A	5 A

1	f	101	No Abnormalities Detected		X	X	X	X
			Scheduled sacrifice		.	.	.	X
		102	No Abnormalities Detected		X	X	X	X
			Scheduled sacrifice		.	.	.	X
		103	No Abnormalities Detected		X	X	X	X
			Scheduled sacrifice		.	.	.	X
		104	No Abnormalities Detected		X	X	X	X
			Scheduled sacrifice		.	.	.	X
		105	No Abnormalities Detected		X	X	X	X
			Scheduled sacrifice		.	.	.	X
2	f	201	No Abnormalities Detected		X	X	X	X
			Scheduled sacrifice		.	.	.	X
		202	No Abnormalities Detected		X	X	X	X
			Scheduled sacrifice		.	.	.	X
		203	No Abnormalities Detected		X	X	X	X
			Scheduled sacrifice		.	.	.	X
		204	No Abnormalities Detected		X	X	X	X
			Scheduled sacrifice		.	.	.	X
		205	No Abnormalities Detected		X	X	X	X
			Scheduled sacrifice		.	.	.	X
3	f	301	No Abnormalities Detected		X	X	X	X
			Scheduled sacrifice		.	.	.	X
		302	No Abnormalities Detected		X	X	X	X
			Scheduled sacrifice		.	.	.	X
		303	No Abnormalities Detected		X	X	X	X
			Scheduled sacrifice		.	.	.	X
		304	No Abnormalities Detected		X	X	X	X
			Scheduled sacrifice		.	.	.	X
		305	No Abnormalities Detected		X	X	X	X
			Scheduled sacrifice		.	.	.	X

Nominal Dose:			Group 1 - 0% Vehicle Control	Group 2 - 5%	Group 3 - 25%			
			Group 4 - 50%	Group 5 - 100%	Group 6 - 25% Positive Control			

Local Lymph Node Assay (LLNA) in Mice

Individual Clinical Observations and Mortality Records

Day numbers relative to Start Date					0	1	2	5
Group	Sex	Animal	Clinical Sign	Site	A	A	A	A
4	f	401	No Abnormalities Detected		X	X	X	X
			Scheduled sacrifice		.	.	.	X
		402	No Abnormalities Detected		X	X	X	X
			Scheduled sacrifice		.	.	.	X
		403	No Abnormalities Detected		X	X	X	X
			Scheduled sacrifice		.	.	.	X
		404	No Abnormalities Detected		X	X	X	X
			Scheduled sacrifice		.	.	.	X
		405	No Abnormalities Detected		X	X	X	X
			Scheduled sacrifice		.	.	.	X
5	f	501	No Abnormalities Detected		X	X	X	X
			Scheduled sacrifice		.	.	.	X
		502	No Abnormalities Detected		X	X	X	X
			Scheduled sacrifice		.	.	.	X
		503	No Abnormalities Detected		X	X	X	X
			Scheduled sacrifice		.	.	.	X
		504	No Abnormalities Detected		X	X	X	X
			Scheduled sacrifice		.	.	.	X
		505	No Abnormalities Detected		X	X	X	X
			Scheduled sacrifice		.	.	.	X
6	f	601	No Abnormalities Detected		X	X	X	X
			Scheduled sacrifice		.	.	.	X
		602	No Abnormalities Detected		X	X	X	X
			Scheduled sacrifice		.	.	.	X
		603	No Abnormalities Detected		X	X	X	X
			Scheduled sacrifice		.	.	.	X
		604	No Abnormalities Detected		X	X	X	X
			Scheduled sacrifice		.	.	.	X
		605	No Abnormalities Detected		X	X	X	X
			Scheduled sacrifice		.	.	.	X
Nominal Dose:					Group 1 - 0% Vehicle Control			
					Group 2 - 5%			
Group 4 - 50%					Group 5 - 100%			
					Group 3 - 25%			
					Group 6 - 25% Positive Control			

Appendix C
Individual Animal Cell Proliferation Data

INDIVIDUAL ANIMAL CELL PROLIFERATION DATA

EXPLANATORY NOTES

ABBREVIATIONS:

dpm - disintegrations per minute

Local Lymph Node Assay (LLNA) in Mice

Individual Animal Cell Proliferation Data

Animal dpm

Female, 1 - 0% Vehicle Control

101	484.50
102	1140.50
103	891.50
104	746.50
105	1224.50

Female, 2 - 5%

201	927.50
202	705.50
203	879.50
204	787.50
205	448.50

Female, 3 - 25%

301	1263.50
302	1007.50
303	625.50
304	1253.50
305	1232.50

Female, 4 - 50%

401	1219.50
402	344.50
403	2297.50
404	964.50
405	515.50

Female, 5 - 100%

501	472.50
502	1223.50
503	435.50
504	980.50
505	728.50

Female, 6 - 25% Positive Control

601	8892.50
602	6796.50
603	6663.50
604	5317.50
605	5465.50